Record of *Evlachovaea* sp. (Hyphomycetes) on *Triatoma sordida* in the State of Goiás, Brazil, and Its Activity Against *Triatoma infestans* (Reduviidae, Triatominae)

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ABSTRACT A fungal isolate was detected on a dead *Triatoma sordida* (Stål) collected in a peridomestic area in central Brazil. The fungus belongs to *Evlachovaea* Borisov and Tarasov, a new genus that was recently described in Russia. The isolate seems to be a third species and the second new and undescribed species from Brazil. The fungus was shown to be active against *Triatoma infestans* (Klug) third-instar nymphs at a humidity close to saturation. However, activity was reduced at a lower humidity (75%). Values of LC_{50} varied between 1.1×10^5 and 1.5×10^4 conidia/cm^2 treated surface, 15 and 20 d after fungal application and incubation at humidity close to saturation. This new fungus may have a potential for biological control of peridomestic Chagas' disease vectors during the rainy season.

KEY WORDS *Evlachovaea*, *Triatoma sordida*, Chagas' disease, vector control, entomopathogenic fungi

DESPITE SUCCESSFUL CONTROL of triatomine vectors, especially in the southern cone countries of Latin America, Chagas' disease is still a serious health problem in many locations. *Triatoma infestans* (Klug), a typical domestic vector species, has almost disappeared in many regions of Brazil, Uruguay, and Chile. Other species that persist in peridomestic foci, where conventional spraying with synthetic insecticides is less effective, may invade houses and transmit *Trypanosoma cruzi* (Chagas). Natural antagonists, such as predators, parasitoids, or pathogens, reviewed by Ryckman and Blankenship (1984) could potentially be used for control of peridomestic vector populations and diminish the risk of reinfestation of insect-free houses. Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin have been considered as potential control agents for biological control under laboratory conditions (Romaña and Fargues 1987, Luz et al. 1998a, c, Lecuona et al. 2001). However, there is practically no information published about pathogenic fungi occurring naturally on triatomine vectors. Parameswaran and Sankaran (1979) isolated a *B. bassiana* strain from *Linshcosteus* sp. Distant in India. This isolate was shown to be highly active against *Triatoma rubrofasciata* (De Geer) (Parameswaran and Sankaran 1979) and *Rhodnius prolixus* (Luz 1994). Moreover, C. L., L.F.N.R., and G. V. Nery (unpublished data) recently isolated *B. bassiana* and *M. anisopliae* in substrates collected in the state of Goiás, Brazil, in peridomestic habitats that were infested with *Triatoma sordida* (Stål). This species is an important peridomestic vector of Chagas' disease in central Brazil (Lustosa et al. 1984, Diotaiuti 1993). More information about natural occurrence of entomopathogenic fungi in vector-infested areas is important for a better understanding of fungal dynamics and their interactions with the target host insects. We report here the isolation of an entomopathogenic fungus which was detected on a dead specimen of *T. sordida* and describe its activity against *T. infestans* nymphs.

Materials and Methods

In the rural proximity of three municipalities, Morrinhos, Formosa, and São Luis de Montes Belos, in the state of Goiás, Brazil, dead specimen of triatomine bugs were collected in peridomestic and domestic areas during the year of 2001. Cadavers were identified and stored at room temperature (25°C) in plastic tubes (25×40 mm). The bottom of each tube was filled up to 10 mm with dried silica gel (SiO_2), and the crystals were fixed with sterile cotton. Dead insects were placed individually on the cotton, and the tube was hermetically closed. In the laboratory, cadavers were dipped in 93% alcohol, incubated for 3 min in 5% sodium hypochlorite, washed three times in sterile distilled water, and placed on filter paper in a petri dish and incubated in a test chamber ($33 \times 37 \times 22$ cm) at a humidity close to saturation and 25°C. Fungal emergence on cadavers and conidiogenesis were recorded

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daily for 15 d. Experiments for pathogenicity and activity were carried out with laboratory mass-reared *T. infestans*, which originated from Parana state, Brazil. Insects were blood fed on chickens every 2 wk and held at 25 ± 0.5°C, 75 ± 5% RH, and a photoperiod of 12:12 (L:D) h. They were not fed during the assays. To test fungal pathogenicity for triatomines, three unfed, recently molted third-instar nymphs of *T. infestans* were exposed for 1 h to cadavers on which fungal development had been observed within 15 d after incubation. Nymphs were then incubated at a humidity close to saturation. Mortality was observed daily, and dead insects were treated as discussed above. Fungi that developed on dead *T. infestans* nymphs were inoculated on chloramphenicol-added complete medium (CM: 0.001 g $FeSO_4$, 0.5 g KCl, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 6 g $NaNO_3$, 0.001 g $ZnSO_4$, 1.5 g hydrolyzed caseine, 0.5 g yeast extract, 10 g glucose, 2 g peptone, 20 g agar, 0.5 g chloramphenicol, and 1,000 ml distilled H_2O) and incubated for 15 d at 25 ± 0.5°C, 75 ± 5% RH, and a photoperiod of 12:12 (L:D) h. Entomopathogenic fungi were identified microscopically based on morphological reproductive structures.

For tests about activity, isolates with proven pathogenicity for *T. infestans* were cultured as mentioned before, after passage on *T. infestans* nymphs for 10–15 d on CM. Conidia were harvested directly from the surface of these cultures by scraping. Inocula were suspended in 10 ml of sterile 0.1% Tween 80. After filtration of the suspension through hydrophylic cotton, concentrations of conidia were adjusted to 3.3×10^6, 10^7, 3.3×10^7, 10^8, 3.3×10^8, and 10^9 conidia/ml. Ten recently molted and unfed third-instar nymphs were treated by directly spraying 5 ml of each concentration with a Potter spray tower (Burkard, Hertfordshire, United Kingdom). Final deposit of conidia/cm^2 was determined by spraying suspended conidia at 3.3×10^6 and 10^7 conidia/ml, as mentioned before, on sterile cover slips (18 × 18 mm), transferring in 10 ml sterile 0.1% Tween 80, and inoculating 100 μl on chloramphenicol-added CM. The number of colony forming units (CFUs) was checked for 5 d after inoculation. Each CFU was counted as a conidia. Control insects were treated with 0.1% Tween 80 only. After drying for 1 h at room temperature and the specific humidity, each lot of 10 nymphs was placed on filter paper in plastic petri dishes (90 by 15 mm) and transferred to a test chamber (33 × 37 × 22 cm) at 75% RH and humidity close to saturation and then incubated at 25 ± 0.5°C and a photoperiod of 12:12 (L:D) h. Humidity of 75% inside the test chamber was regulated with a saturated solution of NaCl (Winston and Bates 1960). Triatomine bugs, which may persist for long periods without alimentation (Luz et al. 1998b), were not fed during the assays. Mortality of nymphs was monitored daily, and dead insects were stored on dried silica.

Lethal concentrations to kill 50% and 90% (LC_{50} and LC_{90}) were calculated by probit analysis (SAS Institute 2000).

Results and Discussion

A total of 64 dead triatomine specimens, nymphs, and adults of *T. sordida* (96.8%), *Triatoma costalimai* Verano and Galvão (1.6%), and *R. neglectus* Lent (1.6%) were collected in domestic and peridomestic areas of the three municipalities. The relative low amount of detected cadavers was probably because of the fast degradation of dead insects. During field trials in central western Brazil, Luz et al. (1999) observed that dying or dead *T. infestans* nymphs were carried rapidly away by ants. This was also found for dead *T. sordida* after application of *B. bassiana* under field conditions in the same region (C. L., L.F.N.R., G. V. Nery, B. P. Magalhães, and M. S. Tigano, unpublished data). Extrategumental development of fungi was observed on 67.2% of the dead insects. However, only one isolate induced mortality of *T. infestans* third-instar nymphs 10 d after exposure to a mummified *T. sordida* fourth-instar nymph cadaver, which originated from Formosa. Further morphological identification of the fungus revealed the isolate to be a species of *Evlachovaea*, a new genus of hyphomycetic fungi that was described recently in Russia by Borisov and Tarasov (1999). The whitish fungus showed to have conidia that was different in shape and chlamydospores that were markedly different in shape from those of *E. kintrischica* Borisov and Tarasov; this seems to be a third species and the second new and undescribed species from Brazil (R.A.H., C. L., and S. B. Alves, unpublished data).

The high number of saprophytic fungi detected on dead insects may be related to the absence of entomopathogenic fungi or other causalities for insect death. In areas with confirmed vector infestation, synthetic insecticides are generally applied. Insects that died after spraying may persist more time in the habitat because of the elevated content of insecticide. On those cadavers, a high amount of saprophytic, but no entomopathogenic microorganisms, may be found. Entomopathogenic fungi will compete with saprophytic fungi and other microorganisms on cadavers. With an increasing delay between insect death and development of entomopathogenic fungi on cadavers under appropriate conditions, cadavers may suffer progressive decomposition, and the outgrowth of entomopathogenic fungi that caused insect death may be masked by the growth of saprobes or other contaminants. False-negative results of the occurrence of entomopathogenic fungi on studied dead triatomines consequently cannot be excluded. C. L., L.F.N.R., G. V. Nery, B. P. Magalhães, and M. S. Tigano (unpublished data) observed initial growth and conidiogenesis of *B. bassiana* on *T. sordida* cadavers that had been collected in a field trial with the *B. bassiana* strain CG14 (Embrapa). Brought to the laboratory after incubation under field conditions, *B. bassiana* could no longer be detected on cadavers, but other fungi, which were not pathogenic to *T. sordida* (C. L., L.F.N.R., G. V. Nery, B. P. Magalhães, and M. S. Tigano, unpublished data), could be detected.

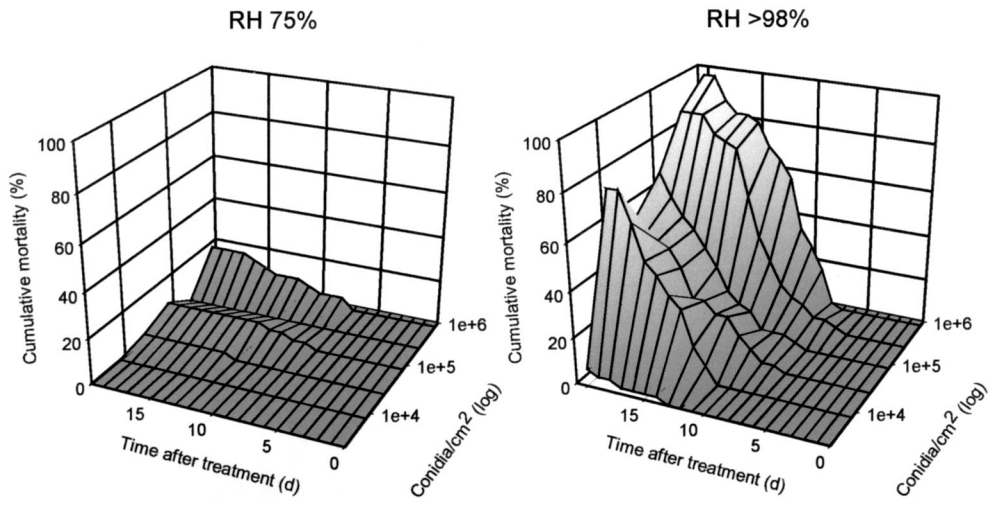

Fig. 1. Cumulative mortality of *Triatoma infestans* third-instar nymphs after application of *Evlachovaea* sp. at different concentrations and incubation at 25°C and relative humidities of 75 and >98%.

Tests about the *Evlachovaea* sp. isolate showed high activity against *T. infestans* third-instar nymphs at humidity close to saturation. First mortality of nymphs was observed 6–8 d after application of *Evlachovaea* sp. at $\geq 3.3 \times 10^7$ conidia/ml and reached 97.5% at highest concentration within 20 d (Fig. 1). Values of LC_{50} and LC_{90} 15 d after treatment were 1.1×10^5 and 1.9×10^6 conidia/cm^2, respectively, and values declined to LC_{50} 1.5×10^4 and LC_{90} 2.2×10^5 conidia/cm^2 20 d after treatment (Table 1). At 75% RH, activity was distinctly reduced, independent of the number of conidia applied (Fig. 1), and it was not possible to calculate the values of LC_{50} and LC_{90} (Table 1).

Humidity was shown to be a key factor in the development of *B. bassiana* on the cuticle of *R. prolixus* (Luz and Fargues 1998, 1999, Fargues and Luz 1998, 2000), and it seems that the activity of the *Evlachovaea* sp. isolate also depends mainly on humidity. Microclimatic conditions in the insect habitats are of considerable importance. Many triatomine species, including *T. infestans*, are predominantly found in regions with semiarid climate and humidity in domestic and peridomestic ecotopes infested by triatomines and can decrease during the dry season as shown by Vasquez-Prokopec et al. (2002) in Northern Argentina. Optimal fungal development on insects can be expected during the wet season, with prolonged nocturnal periods of elevated humidity (Luz 1994), when abundant precipitations are frequent in many regions. Moreover, Luz et al. (1998c) demonstrated a strain-dependent virulence of *B. bassiana* and *M. anisopliae* against *T. infestans* that was related to the relative humidity. There might exist strains of *Evlachovaea* sp. that maintain activity at lower humidities.

The *Evlachovaea* sp. isolate proved to be active against *T. infestans* at conditions of elevated moisture, and this new species may, therefore, be of potential use for controlling peridomestic triatomine vector populations during the rainy season.

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Table 1. Lethal concentrations (LC_{50} and LC_{90} conidia/cm^2) and respective confidence intervals (95% CI) of *Evlachovaea* sp., calculated for *Triatoma infestans* third-instar nymphs, 15 and 25 days after exposure at 75 and >98% relative humidity and 25°C[a]

Humidity tested	Time after inoculation (days)			
	15		20	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
75%	[b]	[b]	[b]	[b]
>98%	1.1×10^5 (3.4×10^4–5.5×10^5)	1.9×10^6 (4.1×10^6–3.4×10^8)	1.5×10^4 (9.0×10^2–3.1×10^5)	2.2×10^5 (3.9×10^4–7.9×10^{12})

[a] Five milliliters suspended conidia at six doses between 3.3×10^6 and 10^9 conidia/ml, corresponding to between 2.4×10^3 and 8.0×10^5 conidia/cm^2-treated surface, were applied on 10 recently molted and unfed individuals each using a Potter spray tower. Assays were repeated four times.

[b] Cumulative mortality insufficient to calculate $LC_{50/90}$.

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